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Colouring matter of various animals, & espe[cially]
deep-sea forms dredged by H.M.S. Challenger

On the Colouring Matters *of* Various Animals, *and especially of* Deep-sea Forms *dredged by* H.M.S. Challenger. By H. N. Moseley, M.A., Fellow of Exeter College, Oxford; late Naturalist on board H.M.S. Challenger. (With Plates I and II.)

During the voyage of the Challenger I made a continued series of observations with the spectroscope on the colouring matters of the various Invertebrata procured by the dredge and trawl. The colours were examined spectroscopically in almost all cases in which an animal presented marked coloration, but usually further attention was only paid in those instances in which a spectrum presenting isolated bands was obtained, such colouring matters being of most immediate interest because they are able to be readily identified.

Observations were also carried on on the colouring matters of shallow water-forms and land animals in continuation of similar work commenced before the voyage was determined on.

A simple direct vision spectroscope by Baker, of High Holborn, was made use of, consisting of slit, collimator, and compound prism only. The instrument was usually made use of without a microscope. The position of the bands was determined by reference to the solar lines, matters being so arranged that half the field of view was occupied by the solar spectrum, whilst the other half showed the absorption spectrum to be determined.

The following are the observations made on various colouring matters.

Sponges.

Poliopogon amadou.—A large Hexactinellid sponge, *Poliopogon Amadou* (Wyville Thomson), which was dredged in 630 fathoms off the Kermadec Islands, showed a bright pinkish-purple colouring of its sarcode. The colour appeared to become developed more vividly on the exposure of the sarcode to the air. The colouring matter is soluble in dilute alcohol and fresh water, but not in absolute alcohol. The solution gave no absorption bands.

Alcyonarians.

Tubipora.—The red colouring of the corallum of Tubipora absorbs all the spectrum but the red, but shows no bands.

Heliopora. — The intense blue colouring of *Heliopora cærulea* is soluble in the recent or dried condition of the corallum, neither in hydrochloric acid, ammonia, caustic potash, nor alcohol. When the corallum is dissolved in hydrochloric acid the blue colouring matter is set free as small flocculent intensely coloured masses, which may be seen under the microscope to be insoluble in concentrated hydrochloric acid. The colouring matter, however, when thus set free is readily soluble in alcohol, and yields an intensely blue solution of the colour of sulphate of copper solution. This solution gives no absorption bands, but simply absorbs most of the red and violet of the spectrum (Pl. I, No. 1). On the addition of alkalies to the solution it becomes of a dirty green colour. It regains its blue colour on being again rendered acid with hydrochloric acid. The blue solution when evaporated to dryness leaves a blue amorphous powder behind. The colouring matter is destroyed by nitric acid.

Zoantharians.

A species of *Anthea,* obtained off Bermuda in 31 fathoms, was of a dark red colour. The red colouring matter gave no absorption bands, but yielded the spectrum shown in Pl. I, fig. 2.

Polyperythrin.—A large series of simple stony corals of very different genera and two forms of Actiniæ, procured mostly from deep water in various localities, and also certain Hydroids, have been found to have a partial or entire deep madder-red coloration, due to the presence of a peculiar colouring matter for which the name "polyperythrin" is proposed. The madder colouring matter yields in the fresh condition a very well-defined spectrum of three absorption bands (Pl. I, fig. 3 *a*), the two less refrangible bands being very dark. One band lies in the green and two at the less refrangible end of the spectrum (Pl. I, fig. 3 *a*). The green band is the faintest and disappears first on increase of light transmitted or diminution of the thickness of the film of colouring matter employed. This banded spectrum is yielded by fresh portions of the ectoderm or any tissues showing the madder coloration. The colouring matter is very stable, and portions of tissues containing it when dried on a glass slide yield the spectrum in full intensity after a lapse of three years. The colouring matter is insoluble in

water, glycerine, alcohol, and ether, also in strong solutions of ammonia or potash, and it is not affected by picric acid. When treated with moderately strong hydrochloric, nitric, or sulphuric acids the colouring matter is dissolved and yields on addition of water or alcohol a solution of a peculiar pink colour, which is markedly dichroic, appearing green in certain lights. This acid pink solution yields when very strong a broad intensely black band, which includes the D line, and which is continued towards the violet end of the spectrum as a less intense additional shading. When the solution is slightly weakened a narrow interval of light divides the black band into two (Pl. I, fig. 3 *b*), and when it is still further reduced in strength the interval between the bands becomes wider; the more infrangible band becomes much more intense than the narrower one on its red side, and the fainter shading on the violet side of the more refrangible band is lost (Pl. I, fig. 3 *c*). When the solution is very weak indeed one band only, the more refrangible of the two, remains. On addition of alkalies to the acid alcoholic solution the colouring matter is precipitated as a dark burnt sienna-coloured flocculent deposit which yields the original three-banded spectrum of the fresh substance, and is redissolved by acids. The precipitate can be separated by a filter and the colouring matter thus obtained in the pure condition. When dried it appears as an amorphous brown powder, which appears to have all the properties of the recent colouring matter.

Polyperythrin was first observed in a species of *Ceratotrochus* (*C. diadema*, n. sp., H. N. M.), which was dredged on July 10th, 1873, between the Azores and Madeira. It appears to be constantly present in various species of the genus *Flabellum*, and gives the red tint which is present in the calcareous corallum of many of these, and which is unimpaired by maceration of the corallum in strong caustics. A large series of *Flabellum variabile* (Semper) was obtained by the Challenger in the Arafura Sea in 60 fathoms. Some of the corals had their soft tissues uniformly coloured of a dark madder. In others the colouring matter was present only in more or less marked streaks, whilst very many specimens were entirely devoid of pigment, and of a uniform white, in fact albinos.

In most of the specimens the corallum was tinged with the colouring matter, but in some it was pure white.

Polyperythrin has been observed in the following Cœlenterata.

Ceratotrochus diadema, dredged off Pernambuco, Brazil,

in 675 fathoms, July 10th, 1873. Ibid., July 10th, 1873. Lat. 37°26′ N., long. 25°14′ W., 1000 fathoms.

Flabellum variabile (Semper), Arufura sea, 40 fathoms.

Flabellum sp., off Cebu, Philippines, 100 fathoms.

Flabellum sp., April 4th, 1874, 120 fathoms.

Flabellum, September 26th, 1874, off Tion Folokker Islands, 126 fathoms.

Fungia symmetrica, occurring at great depths in all parts of the world.

Stephanophyllia formosissima, off Cebu, Philippines, 100 fathoms.

Stephanophyllia sp., Lat. 33°31′S., long. 74°43′W., 2160 fathoms.

Actinia with a coriaceous test, off Japan, 565 fathoms.

Discosoma sp., lat. 33°42′ S., long. 78°18′ W., 1375 fathoms.

Rhizostomean Acaleph (*Cassiopeia*), found in the trawl used in 2040 fathoms, February 11th, 1876, in lat. 42°31′ S., long. 36°27′ W.

Further, several species of Rhizostoma occurring in our northern seas have a brown colouring matter which is probably polyperythrin, but for the examination of which opportunity has not presented itself to me.

In the deep sea actinia obtained off Japan, the outer surface of the animal was of a pure white, and the colouring matter was found in the body cavity only intensely tinging the surfaces of the mesenteries and viscera. In the discosoma the colouring matter occurred in bands or streaks on the disc only. In the Rhizostomean, which was unfortunately too much mutilated for identification, the whole of the surface of the umbrella was thickly covered with the pigment. A second similar acaleph was obtained in the South Atlantic likewise coloured with polyperythrin, but was like the other specimens very much torn.

Polyperythrin has not been met with in any red coloured compound corals nor in any red Actiniadæ living in shallow water, although many such occurring in various parts of the world have been examined, including the red and pink Hydrocorallinæ (Stylasteridæ). Probably, in the numerous medusæ which have a red brown pigment, the pigment is polyperythrin. I lately saw numerous Rhizostomeans cast up on the Norwegian coast thus coloured, but had not then a spectroscope available.

Adamsia.—An Adamsia obtained off the Philippines in eighteen fathoms adherent to the shell of a Pagurus presented pink stripes on its external integument, which was of

a mottled yellow and brown colour. The pink colouring matter in the fresh condition yielded a single well-marked absorption band (Pl. 1, fig. 4). The colouring matter when dried retained its spectrum. It was insoluble in absolute alcohol. The filaments emitted from the pores in the body-wall of these Actiniæ were of a light red colour. This colouring matter gave two absorption bands in the green which were unfortunately not mapped.

Cœnopsammia.—A Eupsammid coral (Cœnopsammia sp.) is extremely abundant about tide mark in sheltered bays at St. Vincent, Cape Verdes, attached to the rock in masses, and very conspicious from its red colour. Some specimens are yellow, and some, half red, half yellow, were met with. Also a red specimen with a yellow bud. The red colouring of the corallum gives an absorption band, the position of which was not determined.

ECHINODERMS.

Purple Pentacrinin.—Many specimens of several species of Pentacrinus were obtained in various parts of the world by H.M.S. Challenger. Several of these species are new, and will be described in due course by Professor Sir C. Wyville Thomson.

Spectroscopic observations were made on specimens obtained on five different occasions and localities, viz.

Off the Kermadec Islands, July 14th, 1874, from 630 and 650 fathoms.

Off the Ke Islands, September 26th, 1874, from 126 fathoms.

Off Cebu, Philippines, January 26th, 1875, from 100 fathoms, with Euplectella aspergillum.

Between Panglao and Siquijor Islands, Philippines, January 25th, 1875, from 375 fathoms.

Off the Meangis Islands, February 10th, 1875, from 500 fathoms.

The majority of the specimens were found to yield a colouring matter which is extremely well defined by characteristic absorption spectra, and which may be termed Pentacrinin, having as yet, been observed only in the genus Pentacrinus. In the fresh condition the colouring matter is freely soluble in slightly acidified alcohol, and gives a solution which is of an intense pink colour when viewed by transmitted light. The solution when moderately intense gives a spectrum consisting of three bands (Pl. 1, fig. 5 *a*). One of these, intensely black with sharply defined margins, covers the D line extending for a very short distance beyond the more refrangible side of that line, and for a greater distance on its other side towards the red, as was

observed by viewing the spectrum with the bright sodium line in the field. The second, situated between D and E, is most intense on its least refrangible border, and shades off towards the violet. The third band is a broad, dim one, stretching from *b* to F. Very little of the red end of the spectrum is cut off. The whole of the violet is absorbed. In very intense solutions (Pl. I, fig. 4*b*) the absorption at the violet end spreads up to *b*, and unites with the third band, so that the light is entirely cut off up to *b*, where there is a defined dark edge. The second band is intensified and becomes black. The first band is black as before, but a paler band is added to it in continuation on its red side. In very intense solutions the two principal bands broaden out and almost run together, being separated only by a narrow interval of yellow. The two principal bands coincide very nearly with those of turacin. The only difference is that the least refrangible band in turacin extends a little further to the green side of D than does that of Pentacrinin.

On the acid alcoholic solution being rendered alkaline by addition of ammonia the solution becomes of a bluish-green colour, which shows a slight red fluorescence on the concentration of sunlight in it.

The spectrum is changed. An intensely black band now occupies a space to the less refrangible side of B; to the red side of D is a broad pale band, whilst the third band between *b* and F remains as before, excepting that it is intensified for rather more than half its breadth on its violet side (Pl. I, fig. 5 *c*). In very weak solutions the first and third bands remain visible after the middle one is lost.

The solution can be rendered acid or alkaline any number of times with corresponding change of colour and spectrum. By careful addition of ammonia in small quantities to the acid solution, in a tall vessel, one part of the solution can be obtained green, whilst the remainder is still red.

Thus the passage of one spectrum into the other may be seen at the junction of the two solutions. As the least refrangible band of the acid spectrum fades, it takes the form of a fine black line to the red side of D, with a fainter margin extending just over D towards the green. As the ammoniacal portion of the solution is approached a dark, broad band is seen to extend gradually towards the red from the least refrangible acid band up to the position of the least refrangible margin of the dark alkaline band in the extreme red. The least refrangible margin of the broad band darkens as the least refrangible acid band fades from view, and at length assumes the full intensity of the least refrangible alka-

line band. In a very strong solution, or with weak transmitted light, the broad band appears to extend the entire distance from the alkaline band in the extreme red to D, whilst the entire violet and blue end of the spectrum is absorbed continuously as far as *b*.

By very cautious neutralisation of the solution a green fluid can be obtained, which yields both acid and alkaline spectra showing four bands (Pl. I, fig. 5 *d*). In it the extreme red band of the alkaline solution and the intense band in the region of D of the acid solution are seen of equal intensity, whilst the second band of the acid spectrum is very faint. In the exactly neutral solution thus yielding four bands, acid and alkaline pentacrinin appear to coexist.

The acid solution of pentacrinin, when slowly evaporated and concentrated, yields a precipitate, which, when collected and dried, appears as a dark violet-coloured amorphous powder.

This substance, viewed by transmitted light as adhering to the filtering paper, and rendered transparent with glycerine, yields the same spectrum as the acid solution.

The dried colouring matter is very sparingly soluble in absolute alcohol, but freely soluble in this, on the addition of a few drops of hydrochloric acid. It is not soluble in dilute hydrochloric acid alone, requiring the addition of alcohol to show the spectrum.

The fresh colouring matter is soluble in fresh water, but remains partly suspended, forming a slightly opaque dark purple solution, which gives, when quite fresh, a mixed acid and alkaline spectrum. When the aqueous solution is acidified the colouring matter becomes entirely dissolved, and the fluid becomes clear and transparent, and of a beautiful pink, yielding the same spectrum as the acidified alcoholic solution. When the intense watery solution is rendered alkaline a green flocculent precipitate is formed, and in a very intense solution the two outer bands, the most refrangible and least refrangible, are lost, being encroached upon and included in the general absorption of the ends of the spectrum.

All the specimens of Pentacrinus obtained off the Kermadecs, which were probably of two species, were of a uniform dusky purple colour when brought to the surface, being evidently coloured by acid pentacrinin.

The specimens obtained off the Meangis islands were possibly of four species. Of these, three forms were similarly coloured dark purple, one being especially dark, whilst

the fourth form was of a light pinkish red, and did not contain purple pentacrinin at all.

The specimens of Pentacrinus obtained off the Ke islands, when in the fresh condition, had their stems almost white, and their crowns of a light yellow or light reddish orange, showing no purple coloration at all; and those dredged off Panglao and Signijor islands were almost colourless; nevertheless, when placed in alcohol, they yielded a solution which was deeply coloured of a sap green, and which, when acidified, became of the usual deep pink of pentacrinin. The pentacrinin was thus in these examples, though present in great abundance, entirely masked. It is possible that these Pentacrini would have shown an alkaline reaction in the fresh condition. Unfortunately the test was not made. The pentacrinin would be far less visible in the green alkaline condition.

Red pentacrinin.—The species of Pentacrinus obtained off the Meangis islands, which was of a light pinkish-red colour, gave a simple light-red solution in absolute alcohol. This solution, when very intense, absorbed all the spectrum except a band of light between E and B (Pl. I, fig. 6 *a*). When the solution was weaker it showed a single broad band in the region of E, *b* and F, with some absorption of the ends of the spectrum (Pl. I, fig. 6 *b*). Addition of ammonia reduced the brightness of the colour of the solution, and when added in quantity caused the absorption band to disappear. It did not render the solution at all green. Hence this one species was devoid of pentacrinin, and contained an entirely different colouring matter.

Antedonin.—The various species of Antedon appear to be usually either of a rose colour or of an orange or yellow, running into a yellow brown or of a dark purple. Both the rose or red and yellow colouring matters are freely soluble in alcohol and usually in fresh water. The coloured solutions obtained from a large number of such species dredged by the Challenger were examined, but none of them yielded a characteristic absorption spectrum showing bands. The European species have been similarly found to yield a colouring matter free from bands. In the case of *Antedon rosaceus* all the spectrum but the red is absorbed. I found a purple species occurring at Suez to yield a similar spectrum.

By dredging in from 8 to 12 fathoms in the channel between Somerset and Albany Island, at Cape York, Australia, an Antedon was obtained in abundance which was of a dark purple colour. The colouring matter is insoluble in glycerine, soluble to a large extent in fresh water, and abundantly

soluble in weak spirit, and gives an intense fuchsin-coloured solution. This, when compared with that obtained from a deep sea Holothurian found to contain the same colouring matter (*v. inf.*), is seen to be much redder, but it becomes pinker as diluted with alcohol, and at last quite pink and indistinguishable to the naked eye from that of the Holothurian. The solution when of moderate strength gives a spectrum consisting of three well-defined absorption bands (Pl. I, fig. 9 *a*). On using a very weak solution and gradually strengthening it the least refrangible band, as being the most intense, appears first, and is visible in solutions which appear very slightly tinted indeed to the unassisted eye. The other two bands appear together. The middle band is at first darkest about E. The most refrangible band, which is much less dark than the middle one, is of uniform intensity. Neither the red nor violet ends of the spectrum are much absorbed.

When the solution is very strong all three bands become intense; the violet disappears and all the light, except the red and yellow, becomes very faint. The bands remain as before, except that the middle band now appears of uniform intensity throughout. When the solution is rendered stronger still, the least refrangible band extends gradually up towards D, and the whole of the spectrum becomes absorbed, except a band of light, consisting of red with a little yellow (Pl. I, fig. 2 *b*).

On the addition of hydrochloric acid to the alcoholic solution the colour changes to an orange, and the spectrum now consists of two bands (Pl. I, fig. 7 *c*), one lying to the red side of E, the other to the violet side of *b* and extending to F. These bands are in weak solutions separated by an interval of light. In very intense solutions the bands are joined by the absorption at the violet end of the spectrum, and the spectrum consists of a simple band of red, yellow, and green light (Pl. II, fig. 9 *c*). In solutions of intermediate strength the two bands are connected together by a clouding interrupted by lighter streaks, whilst the more refrangible band is dark just about the region of F, and has its main darkest mass separated from this dark narrow streak at F by a lighter interval.

On the alcoholic solution being rendered alkaline by addition of ammonia, it changes its colour to a deep violet, and a flocculent purple precipitate is formed in it, which can readily be separated by filtration. The precipitate when dried appears as a violet amorphous powder, which is insoluble in alcohol and oil of cloves, and can thus be

rendered transparent and mounted in Canada balsam as adhering to the filtering paper. This precipitate when thus dried yields the same spectrum as it does when suspended in the solution. The spectrum consists of two bands as figured (Pl. II, fig. 7 *d*).

The dried precipitate is insoluble in water, ether, and alcohol, but soluble in acidified alcohol, and then gives the original acid spectrum (Pl. I, fig. 7 *c*), and when this solution is carefully neutralised the original three-banded spectrum returns, but I obtained it only faintly. Though a very large quantity of precipitate was removed by ammonia from a strong and original alcoholic solution, the solution still remained of a vivid colour and gave its three bands. It was only after continued addition of ammonia that the whole of the colouring matter was precipitated.

The colouring matter appears to require a proportionately increased quantity of ammonia to precipitate it, as the solution becomes weaker. Both the dried colouring matter and the alcoholic solution have maintained their colour and properties unimpaired after a lapse of two years.

Hymenaster.—Several new species of this deep-sea genus of Sir C. Wyville Thomson were obtained by the Challenger from deep water. They are of a brilliant scarlet colour when fresh, and the colour is rapidly discharged in alcohol. The resulting deeply coloured solution yields, however, no absorption bands, but a spectrum in which all the light except the red is sharply cut off. Many other brightly coloured star-fish were examined, but with a similar result.

Hoplacanthinin.—Several specimens of an echinoid of the genus Hoplacanthus were obtained by H.M.S. Challenger. They are of a dark madder colour. Observations were made on specimens obtained in latitude 6°48′ N., longitude 122°25′ E. from 800 fathoms, October 23rd, 1874. The colouring matter is freely soluble in alcohol, yielding a madder-coloured solution, which shows in the spectrum two not very sharply defined bands (Pl. II, fig. 8). The colouring matter became precipitated in the alcoholic solution when left to stand in about twelve hours. The absorption bands could not be obtained from the precipitate.

Antedonin from Holothurian.—On Feb. 26th, 1874, in latitude 62°26′, longitude 95°44′ E., in the extreme south of the South Indian Ocean, a large Holothurian, with a gelatinous test, was dredged from 1975 fathoms. The holothurian was about ten inches long, and three or six broad. The animal was of a uniform dark purple colour. The colouring matter present appeared to be identical with that obtained from the Antedon

dredged at Cape York. It was not obtained in so intense a solution as from the Antedon, the coagulation of the gelatinous matter in alcohol no doubt preventing so free a solution. The spectra yielded by the colouring matter are figured (Pl. II, fig. 9, *a*, *b*, *c*, *d*, *e*). In some unimportant respects a difference between the colouring matter of this Holothurian and that from the Antedon was observed. The alcoholic solution of the Holothurian colouring matter absorbs the red and violet ends of the spectrum more than that of the Antedon (Pl. II, fig. 9 *b*), and when acidified with hydrochloric acid in strong solution shows a greater extension of its less refrangible band towards D than does the similar solution from Antedon (Pl. II, fig. 9 *d*). In weak solutions, however, it gives two bands (Pl. II, fig. 9 *c*) coincident with those of the Antedon. It is probable that the slight difference observed is only due to the turbidity of the solution of colouring matter from the Holothurian, which is caused by the gelatinous nature of the animal. Specimens of the same species of Holothurian, or very similar forms, were several times dredged by the Challenger in deep water in various parts of the world, and they were found to be similarly coloured.

WORMS.

Land planarians.—At Parramatta, near Sydney, New South Wales, two large species of Rynchodemus are tolerably common, one of which is of a uniform Prussian blue colour, whilst the other is of a uniform red. In the blue species the blue pigment is confined to the superficial structures, and is most abundant in the cells containing the rod-like bodies. It is insoluble in alcohol. It changes when acidified with dilute hydrochloric acid to a red, and is soluble in acidified alcohol. Neither the red nor the blue pigments yield absorption bands in the spectrum. The pigment can be rendered blue or red by being made alkaline or acid any number of times.

It was thought possible that the red species would be found to contain the same pigment as the blue one in the acid condition, since the two species are exactly alike in form and occur together, but such was found not to be the case. The red form contains red pigment which does not turn blue on being rendered alkaline, and which is insoluble in acidified alcohol.

Eteone.—A species of Eteone was obtained from 1127 fathoms, lat. 41·57° N., long. 9·42°, which had a bright

green pigment in its appendages as is the case in allied shallow water forms as Phyllodiee.[1]

Sabella.—A Sabella obtained from 600 fathoms contained chlorocruorin as do shallow-water Sabellæ.[2]

Sagitta.—Several species of Sagitta were obtained, some being very large. The large ones contained an abundant red oily pigment soluble in alcohol, which absorbs all the spectrum but the red and yellow (Pl. IV, fig. 1).

Crustacea.

Crustaceorubrin.—Many deep-sea Decapods of various forms are coloured of an intense scarlet. This is the case both with Schizopods, such as Gnathophausia and Petalophthalmos (Suhm), and in the Peneids and Caridids, which are excessively abundant in deep water. The oily red colouring matter of these Crustacea is soluble in alcohol, and is in time entirely removed by spirit from specimens preserved in it. The resulting red solution gives a single broad absorption band in the green and blue (Pl. II, fig. 11). Addition of hydrochloric acid or of ammonia to the solution does not alter the spectrum. A similar red solution yielding the same spectrum is formed when large quantities of red pigmented surface Entomostraca are preserved in spirit. The colouring matter seems to be identical in the two cases.

Pandarus.—A red colouring matter, probably the same as the above, was detected with the microspectroscope in a Pandarus infesting a Carcharias brachiurus, which was caught off the Kermadec Islands. So small a quantity of the colouring matter was present that the absorption band obtained was faint and not nearly so extensive as that shown by intense solutions from the red decapods. It is probable, however, that the colouring matter is the same (Pl. II, fig. 12).

Mollusca.

Aplysiopurpurin.—An Aplysia is very abundant on the shore at St. Vincent, Cape Verdes. Its habits are described by Mr. Darwin in his Journal.[3] The purple fluid emitted by this mollusc is soluble in alcohol. It yields a broad absorption band in the green and blue, consisting of a darker and a lighter portion in weak solutions (Pl. II, fig. 13 *a*), but entirely black in intense solutions. On the solution being acidified it changes to a beautiful violet, and then

[1] E. R. Lankester, 'Journal of Anatomy and Physiology,' vol. iv, p. 121.
[2] Ibid., 'Quarterly Journal Microscopical Science,' Nov., 1867.
[3] 'Darwin's Journal,' 2nd edition, p. 6.

yields the spectrum shown (Pl. II, fig. 13 *b*). The ends of the spectrum are very little absorbed by either of the two solutions. The colouring matters are evanescent and soon fade.

In Bronn's 'Klassen und Ordnungen des Thierreichs'[1] it is stated that the coloured fluid of Aplysia is, when fresh, purple red. Hydrochloric acid in small quantities intensifies the purple colour of the fluid, in larger, changes it to blue. Huschke says the fluid contains iodine, but the purple colour cannot be derived from this.

An Italian chemist has recently stated that an anilin base is present in Aplysia.

Doris.—A Doris or example of a closely allied genus obtained in lat. 0°33′ S., long. 151°34′ W., from 2425 fathoms, September 6th, 1875, had the surface of its foot coloured of a dark purple, whilst the remainder of the body surface was devoid of such pigment. The purple colouring matter was found to be soluble in alcohol acidified with hydrochloric acid, and both when fresh and in solution gave a spectrum showing two well-marked absorption bands (Pl. II, fig. 14) which resemble those obtained from the acidified solution of the colouring matter of Aplysia.

In the Doris, however, the spectrum of the fresh colouring matter is identical with that of the acidified solution. It is thus possible that the colouring matter exists in the animal naturally in the acid form.

Ianthinin.—Some large specimens of Ianthina were procured on the surface of the North Atlantic, on June 20th, 1873, and afforded an opportunity for the examination of the well-known purple-coloured fluid emitted by those animals, and for corroborating some observations made previously during the voyage.

The colouring matter in question is soluble in spirit, but apparently only to a limited extent, since, if after a number of Ianthinas have remained for a day or so in spirit, the coloured spirit be poured off and fresh spirit substituted, the colouring matter is seen to be shed out, and in a dense cloud, and this spirit rapidly becomes as intensely coloured as the preceding.

The spirit solution is of a pale pinkish-blue colour when viewed by directly transmitted light, but when held so that the light reaches the eye from it obliquely it displays a most brilliant red fluorescence, in this matter resembling greatly Æsculin, which is also blue by transmitted light.

When examined spectroscopically by transmitted light

[1] Bd. III, p. 756.

the alcoholic solution shows three absorption bands (Pl. II, fig. 15 *a*). One, an extremely intense band with well-defined edges, extends from a little beyond the less refrangible side of D to about one third of the distance between D and E. The next band is faint, not so broad as the preceding, and situate a little to the less refrangible side of E. The third band is also faint, but rather darker than the last described; it extends from F towards the red, and shades gradually off towards its red edge. The position of the bands is given in the figure. The least refrangible band is so well marked that it is easily seen with a weak solution; but the two others, being fainter, require strong solution used in considerable thickness, and with not too high a dispersion, to come out well. The red end of the spectrum is cut off as far as almost up to B; the blue end is visible just into the violet.

If a drop of hydrochloric acid be added to the spirit solution the colour changes at once to a clear pale blue, and the spectrum changes to a single band (Pl. II, fig. 15 *b*), dark in the centre, and shading off at the edges, which band is placed nearer the red than the dark band of the alkaline solution, and overlaps the D line towards the green by about one third of its breadth. The original colour and spectrum are restored on the fluid being again rendered alkaline with ammonia.

If an ianthina be pricked and made to discharge its purple into a test-tube containing glycerine the colouring matter is dissolved, and a solution obtained which has a deep violet colour, and gives the three bands like the spirit solution.

If the purple be treated in the same manner with ether a coloured solution is obtained, resembling exactly the spirit one in appearance, fluorescence, and spectrum. The ether does not become very highly tinged, but leaves a coloured residue, which, after the ether is poured off, may be dissolved in absolute alcohol. A blueish solution showing one absorption band is the result.

If the fresh purple fluid be treated with acidified ether a most brilliant dark blue solution is obtained, which is not fluorescent, and which gives the spectrum shown (Pl. II, fig. 15 *c*).

No method by which the colouring matter, which may be called Ianthinin, could be preserved was discovered. All the various solutions mentioned above faded in the course of a week or two, and the ethereal solutions even more rapidly.

Phosphorescence.—The phosphorescent light emitted by three genera of deep-sea *Alcyonarians* was examined spec-

troscopically. The spectra are figured in the accompanying woodcut.

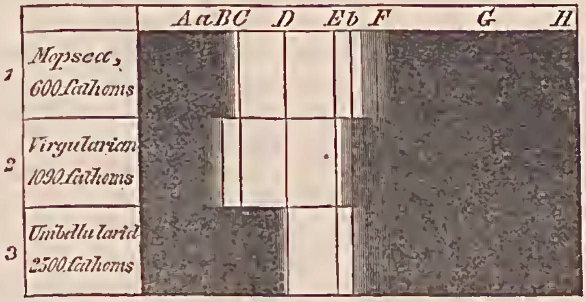


Fig. 1 represents the spectrum obtained from a species of *Mopsea* dredged from 600 fathoms. The light, as usual, was emitted very vividly on the animals being put into fresh water, but, as usual, soon exhausted under the influence of this stimulus.

Fig. 2 represents the spectrum yielded by a *Virgularian* obtained from 1090 fathoms. The yellow was in this case especially brilliant. Portions of phosphorescent tissue were readily detached from the specimen. The light was greatly increased on the tissue being squeezed or rubbed.

Fig. 3 gives the spectrum obtained from an *Umbellula* from 2500 fathoms. Here also the light was greatly increased by the action of fresh water. The specimen was placed first in fresh water, then in salt water, and finally in spirit. Very vivid light was emitted as it came in contact with the spirit. The whole stem was phosphorescent.

It will be remarked that the light emitted by the *Umbellula* consists solely of yellow and green light, whilst both of the other *Alcyonarians* produce a considerable quantity of red light. The *Virgularian* gives out more red light than the *Mopsea;* the *Mopsea,* on the other hand, making up for the deficiency by showing more green.

The effects of various forms of stimulus on the amount of light produced seems in these deep-sea *Alcyonarians* to agree with those to be observed in the case of similar shallow-water forms.

General Remarks.

My friend, Professor Lankester, in his long series of observations on animal colouring matters, published at various times,[1] seems to have been somewhat unfor-

[1] *Blue Stentorin.*—"The Colouring Matter of *Stentor cæruleus,*" by E.

tunate in not meeting with a larger number of these colouring matters yielding characteristic banded spectra. One of the most striking results of the present series of observations is the comparatively large number of animal coloured bodies yielding banded spectra which have been met with. It must, however, be remembered that in the present instance also a very large number of animal colouring matters occurring in animals of most various kinds spread over the greater part of the world have been passed in review with a very large proportion of negative results, and that the results here published represent the fruit of work extending over a period of three years and a half.

Professor Lankester in his paper on Blue Stentorin above cited[1] enumerates the few animal colouring matters known at the time of the publication of his paper to yield banded spectra. The list must now be more than doubled, and should stand thus:

Hæmoglobin (for its distribution see Lankester, 'Proc. Royal Soc,' No. 140, 1873).

Chlorocruorin (Lankester, 'Quart. Journ. Micro. Science,' Nov., 1867, 'Journal of Anat. and Physiol.,' vol. iv.)

Bonellein (Sorby, 'Quart. Journ. Micro. Science,' vol. xv, 166).

Bile-pigments and derivatives.

Chlorophylloid substances (in Spongilla fluviatilis, Sorby, 'Quart. Journ. Micro. Science,' vol. xv, p. 7).

Turacin (A. H. Church, 'Phil. Trans. S. Soc.,' 1869, p. 627).

Aphidein and allies (Sorby, 'Quart. Journ. Micro. Science,' vol. xi).

Actiniochrome, from Bunodes crassicornis (H. N. Moseley, 'Quart. Journ. Micro. Science,' vol. xiii, p. 143, 1873).

Blue stentorin (Lankester, ibid., p. 139).

Pigment of Odax and Labricthys (fish) (George Francis, of Adelaide, 'Nature,' p. 167, Dec. 30th, 1875).

Polyperythrin.

Red colouring of Cœnopsammia.

Two distinct colouring matters in Adamsia sp.

Purple pentacrinin.

Red pentacrinin.

R. Lankester, 'Quart. Journ. Microscopical Science,' Vol. XIII, new ser. See also other papers in the same Journal.

[1] Abstract of a "Report on the Spectroscopic Examination of Animal Substances," presented to the British Association at Exeter, 1869, by E. R. Lankester, 'Journal of Anatomy and Physiology,' vol. iv.

Antedonin, found in certain Holothurians as well as in Antedon.

Hoplacanthinin.

Crustaceorubrin.

Aplysiopurpurin (the same probably in Doris).

Ianthinin.

No doubt the list is capable of much furthur extension. Besides hæmoglobin, the distribution of which is so wide and yet so partial (Lankester, 'Proc. Royal Soc.,' No. 140, 1873), and bile pigments, peculiar colouring matters giving absorption spectra have now been found to exist in members of all the seven groups of the animal kingdom. Amongst Protozoa such colouring matters occurs in Infusoria and Sponges; amongst Cœlenterata they occur both in Anthozoa and Hydromedusæ, in Echinodermata in both Crinoidea, Echinoidea and Holothuroidea, but not in the Asteroidea. In Vermes, in Annelids and Gephyreans. In Arthropoda, in Crustacea and in Insecta. In Mollusks, in Gasteropods only. In Vertebrata, in four fish, three species of Odax, and one Labricthys, and twelve birds[1] of two closely allied genera. The Echinodermata and Cœlenterata appear to be the groups which are most prolific of such colouring matters.

The apparently capricious restriction of these colouring matters to certain parts only of the animals possessing them has been dwelt on by Professor Lankester (*l. c.*). In the case of hæmoglobin such instances, as its restriction to the pharyngeal muscles of certain Gasteropods, and the nerve-ganglia of *Aphrodite aculeata*, may be cited, as also its occurrence only in the muscles of the dorsal fin of Hippocampus amongst the muscles of that fish. I may add an observation of my own of a somewhat parallel case to this latter, viz. that in sharks of the genus Carcharias, of which many were caught and skinned on board the Challenger, a thin layer of muscles next the skin, and closely adherent to it, is tinged of a deep red colour with hæmoglobin, appearing like mammalian muscle, whilst all the deeper layers of muscle forming the main mass of the body are pale and almost white. In a *Carcharias brachiurus* caught off the Kermadec Islands this red layer of muscles was not more than a quarter of an inch in thickness. Mr. Lankester accounts for the presence of the hæmoglobin in the muscles of the dorsal fin of Hippocampus by the special activity of that organ, but such an explanation fails in the case of the shark,

[1] During the voyage of the Challenger I believe I saw a notice in some scientific periodical to the effect that turacin had been discovered in an Australian parroquet. I cannot find the statement again.

the skin being apparently immovable. Moreover, the structure of the skin precludes the idea of its having a respiratory function. I believe that the transparent pelagic fish Plagusia will be found, like Leptocephalus, to be devoid of hæmoglobin. I unfortunately did not test it with the spectroscope, but observed several living specimens to be devoid of red colouring under the microscope. I examined many Planarians for hæmoglobin, but did not find it in any, although it occurs in a parasitic species which I found at Suez in 1872.[1]

Besides hæmogloblin several others of the colouring matters here under consideration occur in curiously restricted regions in various animals. I have described in a former paper[2] the curious restriction of actiniochrome in specimens of *Bunodes crassicornis*, which are decolorised by the action of muddy tidal water, to the gonidial tubercles of the animal. Turacin is restricted to certain feathers and certain parts of feathers only, and in Labricthys the green pigment discovered by Mr. G. Francis is restricted to certain stripes on the body. Polyperythrin was found, as already described, to be diffused generally in the tissues of some of the Cœlenterates in which it occurs, whilst in others it is restricted to certain superficial stripes, and in one Actinia to the surfaces of the mesenteries in the interior of the animal only. In different specimens of the same species, *Flabellum variabile*, in which species it is often present in abundance, it is sometimes entirely absent, sometimes tinges the calcareous skeleton, and sometimes does not.

Pentacrinin and Antedonin seem to be widely diffused in immense quantities through the tissues of the crinoids in which they occur; and Echinoderms generally seem to be characterised by the presence of evenly diffused, abundant and readily soluble pigments.

Those colouring matters which, like those at present under consideration, absorb certain isolated areas of the visible spectrum, must be considered as more complex *as pigments* than those which merely absorb more or less of the ends of the spectrum, since in the latter case the sensation of resulting colour is produced by the action on the eye of an evenly graduated range of light of various refrangibilities, whilst in the former the scale of colours is interrupted at variously placed intervals of darkness, and a more complex mixture of residual colours ensues. By the human eye the finer complexities of colour are not distinguished, and although some

[1] H. N. Moseley, 'Nature,' vol. v, January 4th, 1872, p. 184.
[2] H. N. M., on "Actiniochrome," 'Quart. Journ. Mic. Science,' Vol. XIII, 1873, p. 143.

colours produced by absorption spectra, such as turacin, have a tint which strikes the eye at once as remarkable and peculiar, yet it is impossible to tell beforehand which colouring matters will yield absorption bands in their spectra and which will fail to do so.

It seems improbable that the eyes of other animals are more perfect as spectroscopes than our own, and hence we are at a loss for an explanation on grounds of direct benefit to the species of the existence of the peculiar complex pigment in it. That the majority of species of Antedon should have vivid colouring matters of a simple character and that few or one only should be dyed by a very complex one is a remarkable fact, and it seems only possible to say in regard to such facts that the formation of the particular pigment in the animal is accidental, *i. e.* no more to be explained than such facts as that sulphate of copper is blue.

A certain organic compound becomes formed in the animal or plant in course of evolution, either as a directly serviceable tissue-forming element or gland component, or possibly as an inert and almost exerctory product. And this compound has a complex absorptive action on light. In some animals and plants the coloured compound is turned to account by natural selection,[1] increased in quantity and distributed in various ways, either for sexual adornment, concealment, or possibly in such cases as Actinia for the attraction of prey; in others it remains unused. In some instances a colouring matter may exist in an animal or group as a rudiment, having lost a sexual or other use which it had in the ancestors of the animal in question, but having persisted. No doubt this is the case with the colouring matters of many deep-sea animals. In some cases, again, a complex substance, produced by evolution for strictly physiological purposes, and happening to have a bright colour, may be turned to further advantage by some animals possessing it for beneficial external adornment. This would seem to be the case with hæmoglobin, the redness of which, considered as to the colour only, has no use in the majority of animals, and is indeed mostly concealed in utter darkness; but in some instances, as in the cock's comb and in the faces of the white races of man, is turned to account for sexual adornment. It is quite possible that in such instances as Pentacrinus the very abundant colouring matter (Pentacrinin) may have some important physiological function as yet unknown.

It is remarkable that in animals coloured by most widely different colouring matters albinism should occur in certain numbers of individuals of a species.

[1] Notably the case of chlorophyll in green plants.

When large numbers of *Flabellum variabile* were dredged by us in the Arafura Sea a considerable number[1] were always found to be entirely devoid of pigment and pure white, the corallum itself even being colourless. Similarly amongst 300 or 400 specimens of *Renilla* (*violacea ?*) which were dredged in the mouth of the La Plata, off Monte Video, one specimen was found to be of a pure white, all the remainder being of the deepest violet. In ordinary vertebrate albinos only skin pigments are affected; but in what may be regarded as albino genera, such as the fish *Leptocephalus* and in the pelagic *Plagusia*, even hæmoglobin has disappeared.

Colouring matters must have a pedigree and a developmental history which will, in some instances, be able to be traced in the same manner as that of an organ of the body or an histological tissue. A pigment thus may become developed at the root of a zoological phylum, persist in some branches, die out in others, and in some possibly reappear by heredity. The existence of Polyperythrin in both Actinozoa and Hydrozoa amongst Cœlenterates, and its very irregular but nevertheless wide-spread distribution amongst these, seems to be only explicable on such an hypothesis. It is quite possible that the tracing of zoological relations may be facilitated by the use of the spectroscope. A careful chemical examination of some of these numerous colouring matters which do not in the fresh condition yield banded spectra would, no doubt, give evidence of their being transitional to certain of the more complex colours, which latter might possibly be produced from them artificially by action of reagents.

Most Echinoderms are endowed with intense colouring matters yielding a spectrum, in which nearly all but the red or red and a little yellow is absorbed. Since some few forms in each group of the Echinoderms, except the starfish, have colouring matters yielding banded spectra, and the same colouring matter, Antedonin, occurs in so widely separated forms as Holothuria and Antedon, it is quite possible that in most cases a mixture of colouring matters masks a pigment common to many members of the group, and yielding a banded spectrum. The examination of the colouring of young animals might yield interesting results. At the same time, no doubt, many colouring matters may have had an entirely isolated formation, as in the case of Turacin, to which there seem to be no stepping-stones; and the necessary

[1] It even became a question whether the majority of specimens were not unpigmented, in which case the exhibition of pigment or chromatism—as it might be termed, in antithesis to albinism—would become the exceptional variation in the species instead of the rule.

instability of highly complex chemical substances would render the existence of any but a very imperfect phylum of pigments impossible.

A considerable number of animal colouring matters with banded spectra may be made to yield two different spectra, according as they are rendered acid or alkaline; and they exist in the animals in which they occur either in the acid or alkaline condition, as shown by the spectra. In other animals, as in the case of Antedonin in the Holothurian and the Antedon, the colouring matter has three phases, acid, alkaline, and neutral, and exists in the animals in the intermediate neutral condition.

Colouring Matters of Deep-Sea Animals.—Very little, if any, light can penetrate from the surface of the sea to depths such as 1000 or 2000 fathoms, and I believe that experiment has shown that little or no effect is produced on sensitized paper at the moderate depth of sixty fathoms. It is probably, as far as solar light is concerned, absolutely dark at depths of 1000 fathoms and upwards, and the fact that two blind decapod crustacea were dredged by us in 450 to 490 fathoms (off Sombrero, D. W. I., March 15th, 1873) seems to point to a condition of extreme darkness at much less depths. Nevertheless, several facts show that at these depths light of some kind must exist. Some deep-sea animals are entirely destitute of the eyes possessed by their shallow-water congeners, and appear, like the blind cave animals, to rely on touch alone, being provided with specially long antenna hairs, or fine rays, for the purpose of feeling. Other animals, however, living in very deep water, have enormously enlarged eyes, and hence some light must exist; and a further evidence that such must exist is the fact that several small deep-sea Lophioid fishes have the dangling lures on their heads specially developed, and apparently rendered attractive with a view to enticing their prey, as in the case of the Angler. Professor Sir C. Wyville Thomson and Dr. Carpenter have suggested that phosphorescent animals form the source of light in the deep sea. All the Alcyonarians dredged by the Challenger in deep water were found to be brilliantly phosphorescent when brought to the surface, and their phosphorescence was found to agree in its manner of exhibition with the same conditions as are observed in the case of similar shallow-water forms. There seems no reason why the animals should not emit light when living in deep water just as do their shallow-water relatives. The light emitted by phosphorescent animals is quite possibly in some instances to be regarded only as an accidental

product, and of no use to the animal, although, of course, in some instances it has been turned to account for sexual purposes, and may have other uses occasionally. There is no reason why a constant emission of light should be more beneficial than a constant emission of heat, such as takes place in our own bodies, and it is quite conceivable that animals might exist to which obscure heat-rays might be visible, and to which, therefore, men and mammals generally would appear constantly luminous.

However, be the light beneficial to them or not, it seems certain that the deep sea must be lighted here and there by greater or smaller patches of luminous Alcyonarians, with wide intervals, probably, of total darkness intervening. Very possibly the animals with eyes congregate around these sources of light.

The phosphorescent light emitted by three species of deep-sea Alcyonarians was examined with the spectroscope and found to consist of red, yellow, and green rays only. Hence, were the light in the deep sea derived from this source, in the absence of blue and violet light, only red, yellow, and green colours could be effective. No blue animals were obtained in deep water, but blue animals are not common elsewhere. It is remarkable that almost all the deep-sea shrimps and schizopods, which were obtained in very great abundance, are of an intense bright scarlet colour, differing markedly in their intensity of colour from shallow-water forms, and having apparently for some purpose developed an unusually large quantity of the same red pigment matter which colours small surface crustacea.

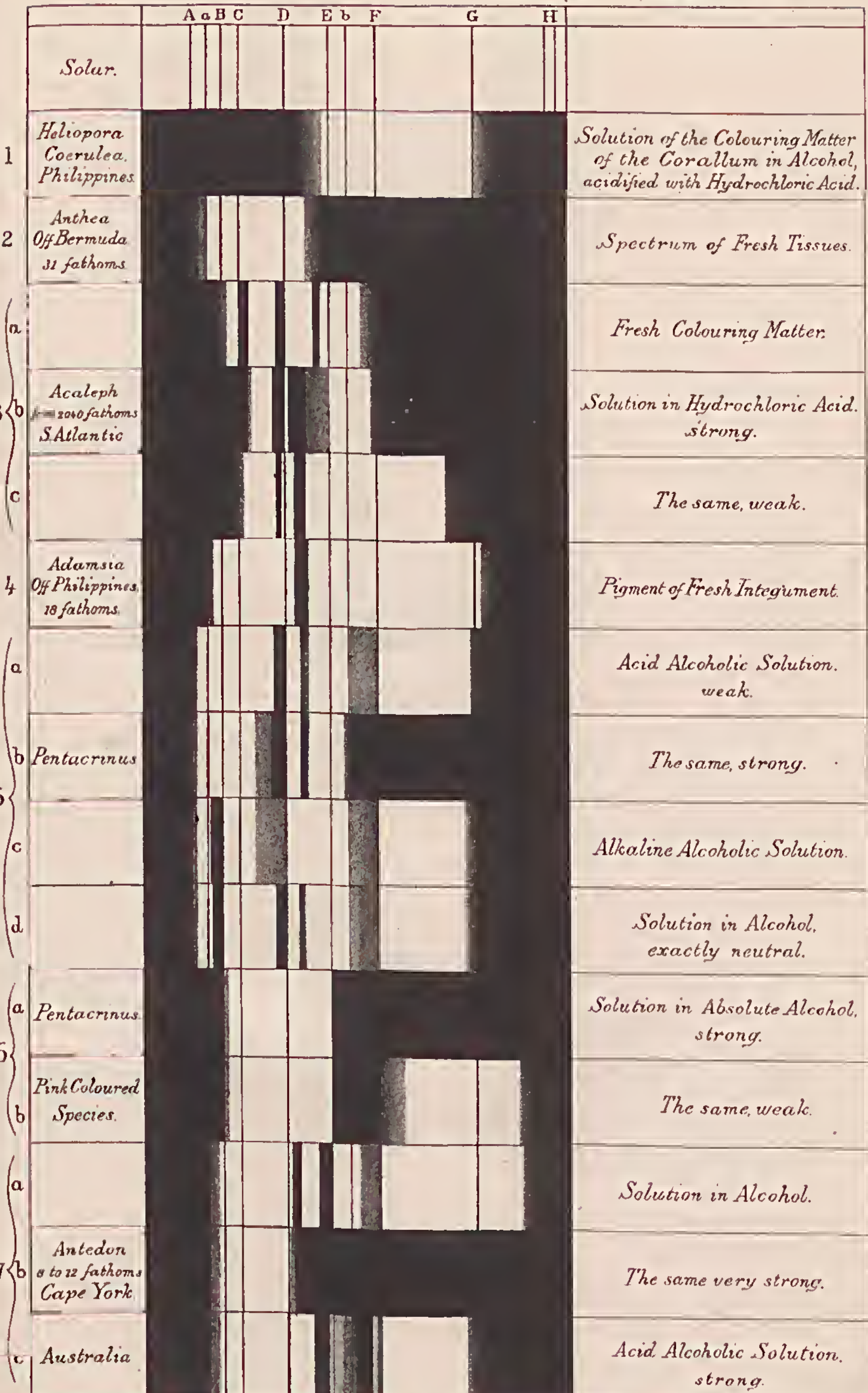
A brilliant green colouring matter was found in some deep-sea Annelids.

No doubt in many cases the colouring of the deep-sea animals, as in the case of the purple Holothurians, is useless and only a case of persistence. The madder colouring of some of the soft parts of the Corals may be in like case, but possibly useful for attraction of prey, being visible by the phosphorescent light. Nearly all, if not all, of the fish certainly living on the bottom in the deep sea were of a dull black or quite white and semi-transparent.

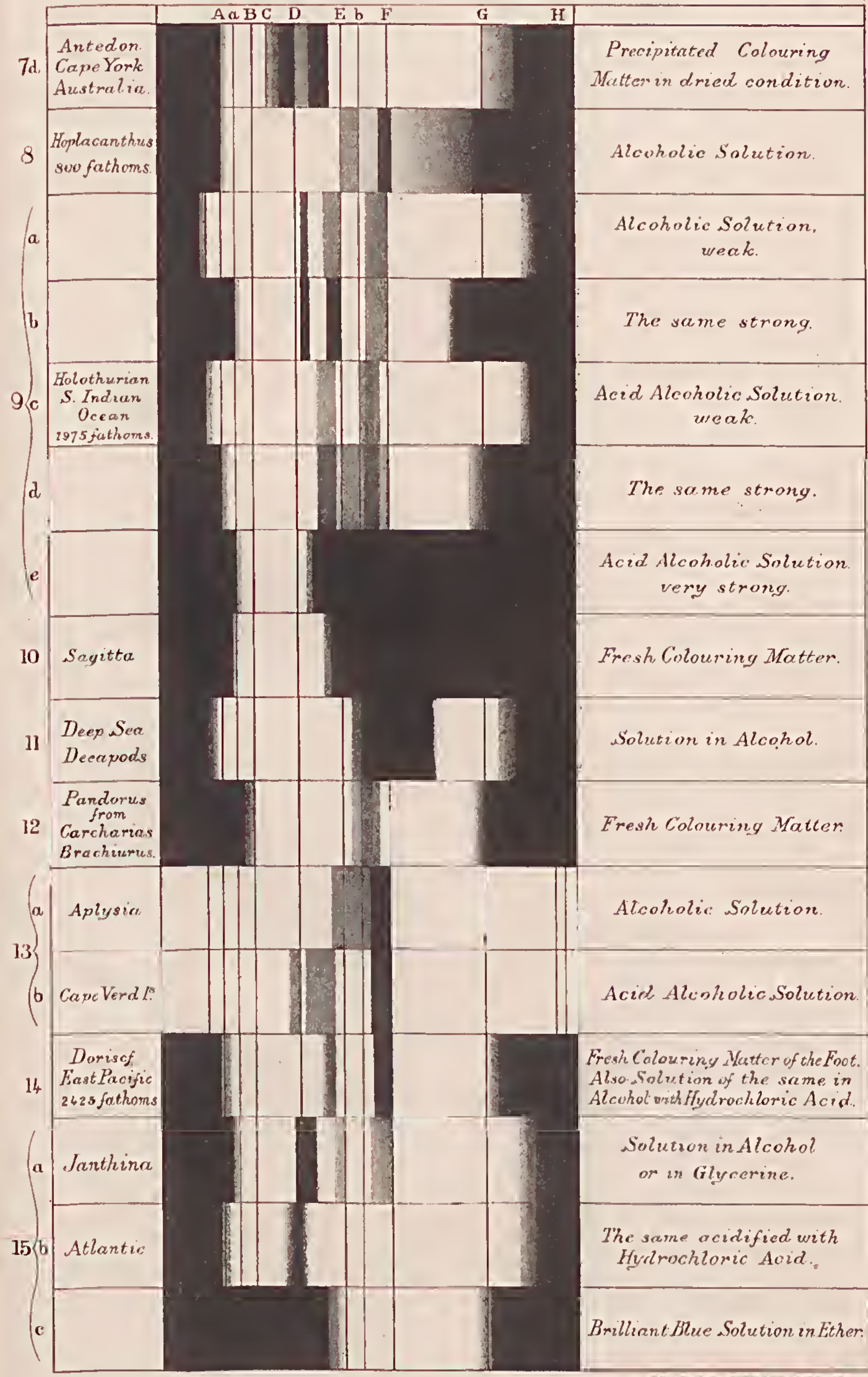
I regret much that I did not examine deep-sea fish with regard to the existence and amount of hæmoglobin to be found in them.

The same colouring matters exist in deep-sea animals which are found in shallow-water forms. Polyperythrin is found abundantly in surface-swimming Rhizostomæ and in deep-sea Corals and Actiniæ. Antedonin occurs in a shallow water

(nine fathoms) Antedon at Cape York and in a Holothurian found in 1975 fathoms near the Antarctic Sea. No doubt in many instances in the case of deep-sea possessors of these pigments, the pigments from being in the dark never exercise their peculiar complex action on light during the whole life of the animal, but remain in darkness, never showing their colour, as does hæmoglobin in so many animals.



M^c. Farlane & Erskine, Lith^rs. Edin^r.



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